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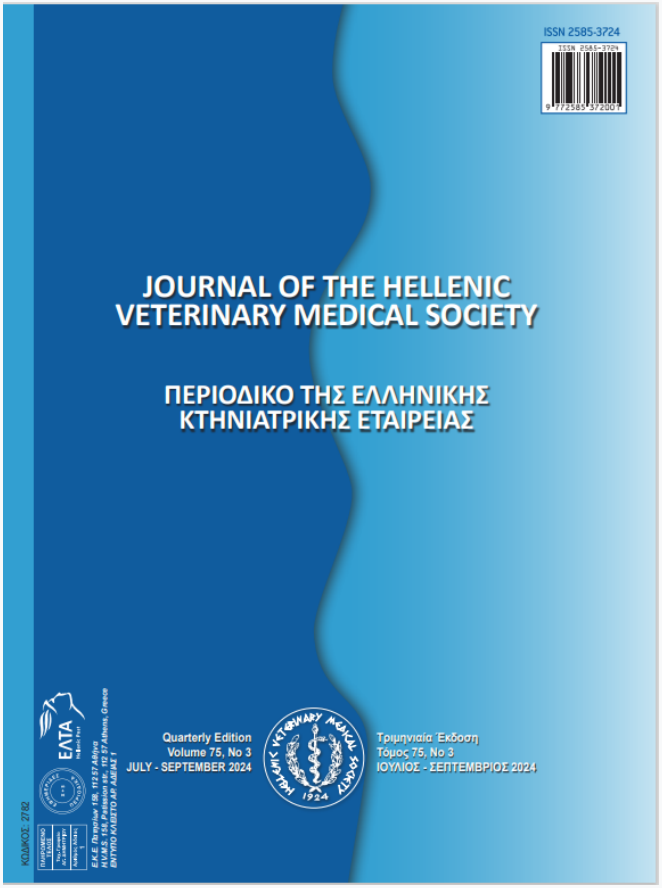

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Impact of Antioxidant Addition to Quail Feed on Progeny's Antioxidant Status and Glutathione Peroxidase 1 Gene Expression

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ABSTRACT: In this study, the effect of antioxidant supplementation in breeder quail feed on the performance, total antioxidant capacity, glutathione peroxidase enzyme activity, and expression level of glutathione peroxidase 1 gene in liver tissue of progeny at hatching and 21-d-old were investigated. A total of 432 Japanese quail (*Coturnix japonica*) breeders (a total of 432 breeders, 324 female and 108 male) were divided into six groups. Breeder quails in the control group were fed with a basal breeder quail ration. Birds in the experiment groups were fed with commercial breeder feed supplemented with 0.35 mg/kg Sel-Plex, 40 mg/kg L-carnitine, 50 mg/kg DL-methionine, 250 mg/kg vitamin E, and 10 mg/kg taurine, respectively. After four weeks of the breeder feeding period, 1200 eggs were placed in the incubator, 200 fertile quail eggs from each group. Hatched chicks were placed in the same trial groups as the breeders and fed with basal quail chick ration. Breeder quails and progeny were fed ad libitum for four weeks. The lowest hatchability of total eggs, fertility, and hatchability of fertile eggs was determined in the taurine group (77.5%, 91.5%, 84.70%, respectively) ($p<0.05$). Mid-term embryo mortality did not occur in the selenium group ($p<0.05$). Body weight, feed consumption, body weight gain, and feed conversion ratio of progeny were not affected by supplementation with antioxidants ($p>0.05$). Supplementation of methionine and vitamin E increased glutathione peroxidase enzyme concentration in liver tissue of chicks at hatching (1.58 ng/mg and 1.88 ng/mg, respectively) and 21-d-old (2.74 ng/mg and 2.72 ng/mg, respectively). The addition of antioxidants to the diet increased the total antioxidant capacity in liver tissue only at hatching ($p<0.05$). Dietary antioxidant supplementation to breeder feed down-regulated GPX1 gene expression in progeny at hatching. As a result, antioxidant supplementation to breeder basal diet affected the hatchability of total eggs, fertility, embryonic deaths, glutathione peroxidase enzyme concentration, total antioxidant capacity, and glutathione peroxidase 1 gene expression levels.

***Keywords*:** Antioxidant; Gene expression; Glutathione peroxidase; Japanese quail; Total antioxidant capacity

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INTRODUCTION

Antioxidants protect cells against oxidative damage (Ramazani et al., 2023). The antioxidant system protects the cell from toxic products of metabolism and free radicals (Ullah et al., 2016). There are thousands of compounds with antioxidant properties in nature (Surai, 2007). Malondialdehyde (MDA) levels are typically used as an indicator of lipid peroxidation (LPO) and are a dependable biomarker of LPO (Acaroz et al., 2018). Enzymes are affected by reactive oxygen species (ROS) and LPO production (Acaroz et al., 2019). Cells are protected against oxidative damage by the antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD), which are important in defending against oxidative stress (Acaroz et al., 2019; Acaroz et al., 2018). During embryo development, the antioxidant content of the egg protects the embryo from the harmful effects of free radicals (Watson et al., 2018). The antioxidant composition of the egg is significantly influenced by factors such as egg weight, genetic structure, breeder age, storage conditions, and the composition and quantity of feed consumed, because these factors change the chemical composition of the egg and potentially impact post-hatching chick/poultry performance and survival (Karageçili and Karadaş, 2017; Nimalaratne et al., 2016; Nimalaratne and Wu, 2015).

About 90% of the energy needed for embryonic growth comes from fatty acid oxidation (Buzała et al., 2015). Thus, the growth of the chicken embryo depends on the aerobic mechanism of β-oxidation of fatty acid chains in the yolk (Noble and Cocchi, 1990). The energy system, which depends on the transportation of nutrients from the yolk for the development of embryonic tissues, requires the consumption of oxygen (Karageçili and Babacanoğlu, 2022). The oxygen consumption of the embryo increases significantly in the middle of the incubation period. High energy metabolism in poultry embryos can lead to the production of free radicals and reactive oxygen species and damage the macromolecules of the embryo cell (Liu et al., 2021). Polyunsaturated fatty acids are quite susceptible to oxidative damage from free radicals (Singh et al., 2019), and the fats in various tissues of the chick embryo are primarily unsaturated (Yigit et al., 2014). Therefore, many antioxidant compounds protect the chick embryo against oxidative damage (Karageçili and Karadaş, 2017).

One of the main natural antioxidant compounds in the animal body is vitamin E (Surai, 2007). During embryonic development, vitamin E is transferred from the egg yolk to the embryo's tissues, where it breaks the cycle of lipid peroxidation to shield the developing organism from the damaging effects of oxidative damage (Niki, 2014; Panda and Cherian, 2014; Surai et al., 2016). It was shown that adding vitamin E to breeder feed increases the amount of vitamin E in developing chick tissues and substantially reduces lipid peroxidation (Surai, 1999).

As a component of selenoproteins such as thioredoxin reductase (TrxR), glutathione peroxidase (GPX), and iodothyronine deiodinase (ID), selenium (Se) is an important element that is crucial for antioxidant defenses (Surai et al., 2016). The Se concentration and form in feed determine the Se concentration in egg yolk and albumen (Jing et al., 2015). A significant increase in Se concentration in embryo tissues is associated with organic selenium content in feed (Kahraman et al., 2020; Muhammad et al., 2021; Yuan et al., 2011; Zhao et al., 2023). The increase in the amount of Se in egg yolk and albumen increases the Se level in the embryonic liver, contributes to the antioxidant defense of the developing chick (Surai and Kochish, 2019), and affects gene expression in the embryo (Surai et al., 2016).

Carnitine (L-carnitine), which acts as an emergency transporter in the beta-oxidation of long-chain fatty acids to produce energy in mitochondria, is synthesized from the essential amino acids methionine and lysine (Agren et al., 2014). Additionally, it helps mitochondrial elimination of short- and medium-chain fatty acids that occur during normal and abnormal metabolic events (Virmani et al., 2013). Carnitine specifically contributes to the passage of lipids across the yolk membrane from the yolk to embryonic tissue (Kucharska-Gaca et al., 2017). Carnitine protects the organism from oxidative stress by firstly scavenging direct free radicals, secondly inhibiting enzymes that form free radicals in the electron transport chain in mitochondria under stress, and thirdly using important transcription factors, including nuclear factor erythroid 2-related factor 2 (Nrf2) and nuclear factor-B (NF-B) to activate a variety of antioxidant enzymes (Surai, 2015). However, it was reported that the amount of L-carnitine in the eggs of chickens fed with plant-based feeds is low (AL-Jomaily and Taha, 2020; Rezaei et al., 2008). In addition, the embryos' ability to synthesize L-carnitine is constrained by inadequate activity of the γ-butyrobetainehydroxylase enzyme, which is crucial for carnitine biosynthesis during incubation (Zhai et al., 2008).

Methionine participates in the synthesis of L-carnitine and also forms the structure of body, egg, and feather proteins (Pack, 1996). An important methyl donor, it is a methyl group donor in enzymatic reactions. Methionine is also involved in synthesizing carnitine and taurine, with its contribution to transsulfuration reactions. In addition, cysteine used in the synthesis of glutathione (GSH) is also synthesized from methionine (Coleman et al., 2019). Hepatocytes take up methionine more readily than cysteine for the production of glutathione (Choppadandi et al., 2019). Glutathione serves a crucial function in detoxification and preserves cells from oxidative damage (Pizzorno, 2014). Furthermore, methionine's thiol group can remove lead from tissues by chelation (Fang et al., 2017). GSH and amino acids containing sulfur are antioxidants (Mosharov et al., 2000).

Taurine is synthesized in the body from methionine and cysteine (Hou et al., 2020). Taurine is high in animal protein sources and almost absent from plant-based feed ingredients (Johnson et al., 2015). Previous studies showed that taurine amino acid has direct antioxidant activity with its oxygen-reactive agent-scavenging property (Cozzi et al., 1995; Redmond et al., 1996) and indirectly contributes to protecting the cell from oxidative damage by reducing membrane permeability (Banks et al., 1992; Geden et al., 1992). Broiler weight gain and feed conversion ratio were positively affected under heat stress when taurine was added to the feed (Hafeez et al., 2021).

This study aimed to examine the effect of antioxidant supplementation (Se, carnitine, methionine, vitamin E and taurine) in the diet of Japanese quail on incubation results and feed consumption (FC), body weight (BW), body weight gain (BWG) and feed conversion ratio (FCR) of progeny and total antioxidant capacity (TAOC), glutathione peroxidase (GPX) concentration and expression level of glutathione peroxidase 1 (GPX1) gene in the liver tissue of progeny at hatching and 21-d-old.

MATERIAL AND METHODS

Animal

A total of 432 Japanese quail (*Coturnix japonica*) breeders (324 female and 108 male quails) and 1008 of their chicks were used in this study.

Chemicals

Antioxidants that were used in the trial were purchased as follows: selenium in organic form (Sel-Plex Alltech, Türkiye), L-carnitine (Lonza Group Ltd., Switzerland), the DL-methionine form of methionine (Kartal Kimya, Türkiye), vitamin E (Kartal Kimya, Türkiye) and taurine (Sigma-Aldrich, Germany). The total antioxidant capacity in liver tissues of quail chicks was assessed using an Elabscience® Total Antioxidant Capacity Assay Kit. Randox® Ransel Glutathione Peroxidase kit was used for glutathione peroxidase enzyme analysis. RNA later (Qiagen), RNeasy Plus Mini Kit (Qiagen), GE Buffer (Qiagen), 5X Reaction Buffer (Qiagen), reverse transcriptase mix (Qiagen), and primer (Primer Array System, UK) were used for gene expression analysis.

Study Design

This study was carried out in the poultry unit of Van Yuzuncu Yil University Animal Research and Application Centre. A total of 432 Japanese quail (*Coturnix japonica*) breeders (324 female and 108 male quails) were divided into six groups and each group was divided into three subgroups (18 females and 6 males in each). Breeder quails were completely randomly distributed into four-tier (95x45x25 cm) battery-type cages according to a completely random design, forming a control group and five experimental groups. Breeder quails in the control group were fed only basal breeder quail diet (Table 1). Animals in the experimental groups were fed with commercial breeder feed by adding 0.35 mg/kg Sel-Plex, 40 mg/kg L-carnitine, 50 mg/kg DL-methionine, 250 mg/kg vitamin E, and 10 mg/kg taurine, respectively. Progeny were fed only with a basal chick diet (Table 1). Breeder quails and progeny were fed ad libitum for four weeks.

After the four-week feeding period of the breeders, 40 eggs (40x5=200 eggs/each group) were collected from each group over 5 days and a total of 1200 eggs were placed in the incubator. Before incubation, eggs were stored at 12 °C and 75% relative humidity. Quail eggs were weighed and numbered, and then a total of 60 eggs (10 from each group) were placed on each incubation tray. The average weight of the quail eggs was 12.05 g in the control group (C), 12.12 g in the selenium group (Se), 11.92 g in the carnitine group (Car), 11.97 g in the methionine group (Met), 12.31 g in the vitamin E group (Vit E) and 11.82 g in the taurine group (Tau). For the first 15 days, the eggs were incubated at 37.7 °C, 60% relative humidity, and 90-degree rotations every two hours. Then, until hatching, the eggs were incubated without rotating at 37.5 °C and 70% relative humidity.

Table 1. Ingredients and chemical composition of basal breeder quail feed and basal quail chick feed

Ingredients	Breeder feed content (g/kg)	Chick feed content (g/kg)
Corn	372.96	342.06
Full-fat soybean -37	381.64	-
Soybean Meal - 47	-	280.00
Sunflower meal -26	96.01	-
Boncalite	-	100.00
Wheat	-	200.00
Soybean oil	-	13.10
Corn gluten	-	30.00
Limestone	66.31	12.24
Blood meal	30.00	-
Poultry meal	30.00	-
DCP	10.27	10.00
Methionine	1.54	0.20
Lysine	-	4.00
Sodium chloride	2.04	3.50
Salmonella preventative	2.00	-
Threonine	0.13	2.40
Carophyllred	1.40	-
Carophyllyellow	0.70	-
Vitamin-mineral premix[1]	2.00	2.00
Toxin trap	1.00	-
Multi enzyme	-	0.50
Sodium bicarbonate	0.50	-
Vitamin E	0.50	-
RovimixHy-D®	0.50	-
Chol Chloride70	0.50	-
Total	1000.00	1000.00
Chemical composition		
Metabolizable energy** (ME), kcal/kg	2900	2900
Dry matter*, %	89.33±0.03	90.39±0.02
Crude ash*, %	10.51±0.06	5.35±0.03
Crude fiber*, %	4.184±0.52	2.943±0.31
Crude fat*, %	6.787±0.07	4.943±0.19
Crude protein*, %	18.066±0.10	21.886±0.06
Calcium**, %	3.30	0.80
Available phosphorus**, %	0.31	0.56
Sodium**, %	0.16	0.20

*Chemical analysis results.
**Calculated value.
[1]Vitamin-mineral premix contains in the following per kg: pantothenic acid, 4000 mg; thiamine, 720 mg/kg; riboflavin, 2 640 mg; pyridoxine, 1 200 mg; nicotinic acid, 12000; biotin, 40 mg folic acid, 400 mg; vitamin K3, 800 mg; vitamin E, 7200 IU; choline chloride, 100000 IU; vitamin D3, 800000 IU; vitamin B12, 6 mg; vitamin A, 3600000 IU, and antioxidant, 40000 mg.

Hatched quail chicks (C= 171, Se= 171, Car=172, Met= 165, Vit E= 174, Tau= 155, 1008 chicks in total) were kept in the same experimental groups, and each group was divided into four replicates and transferred to the chick-rearing cages randomly.

Measured Parameters

FC, BW, FCR, egg weight, and egg production of quail breeders, and FC, BW, and FCR of quail chicks were measured weekly.

At hatching, the hatchability of total eggs, hatchability of fertile eggs, fertility, early mortality, mid-

term mortality, late-term mortality, and under-shell mortality were recorded.

Total antioxidant capacity (TOAC), glutathione peroxidase enzyme activity, and mRNA expression level of the GPX1 gene were analyzed in liver tissue samples (n=8) at hatching and on the 21st day.

The total antioxidant capacity analysis method was performed with a spectrophotometer device following the procedure included in the Elabscience® Total Antioxidant Capacity Assay kit. Glutathione peroxidase enzyme analysis was performed with the ELISA device based on the procedure included in the Randox® Ransel Glutathione Peroxidase kit. The procedure for GPX1 gene expression is given below.

For RNA isolation and cDNA synthesis, 25 mg was taken from liver tissue and preserved in RNA later. The RNeasy Plus Mini Kit was used in the QIACUBE (Qiagen) instrument to isolate RNA, as directed by the manufacturer. A nanospectrophotometer (Thermo, USA) was used to measure the purity of the extracted RNA at 260 and 280 nm. For cDNA synthesis, a total of 10 µl (100 ng/µl) RNA was prepared. After this process, 2 µl GE buffer was added and incubated at 42 °C for 5 minutes in PCR. By adding 4 µl of 5X reaction buffer, 2 µl of primer (Primer Array System, UK), and 2 µl of reverse transcriptase mix, the total amount was increased to 20 microliters. Finally, to activate reverse transcriptase, incubation was carried out at 42 °C for 15 minutes and then at 95 °C for 5 minutes (Abbasi et al., 2011; Önalan, 2019).

Statistical Analysis

The experiment was planned according to the randomized complete block design. The mathematical model is provided below.

$$Yij = \mu + \alpha i + eij$$

Where,

Yij= Observation for the j^{th} repetition of the i^{th} treatment,

µ = Population mean,

αi = Effect of the i^{th} treatment,

eij = Random error associated with the i^{th} treatment and the j^{th} repetition.

The computer package program SAS (2017) was used to analyze data from the study statistically. One-way ANOVA was used to determine the differences between the groups (Tables 2 and 4). Since data at different ages were obtained from different birds in the same group, age and group effects were examined using two-way ANOVA (Figures 1, 2, 3, and 4). In addition, the Duncan test was applied to check the significance of the differences among the groups (Önalan and Seçkin, 2020). The incubation parameter data were analyzed using the Z ratio test (Table 3).

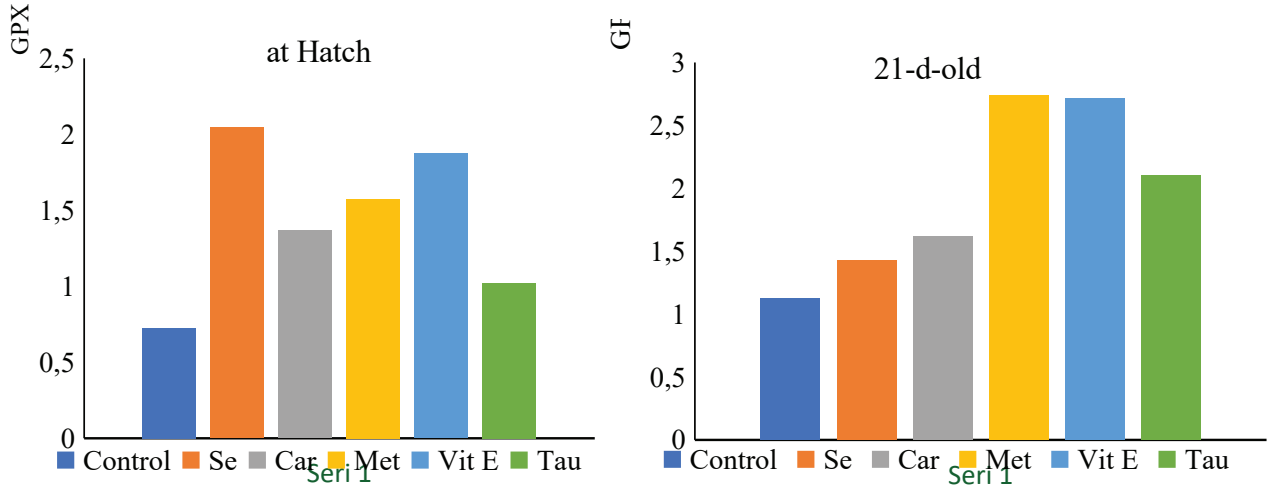

Figure 1. GPX concentration in liver tissue of progeny at hatching and 21-d-old. [abc]Different letters are significant ($p<0.05$).

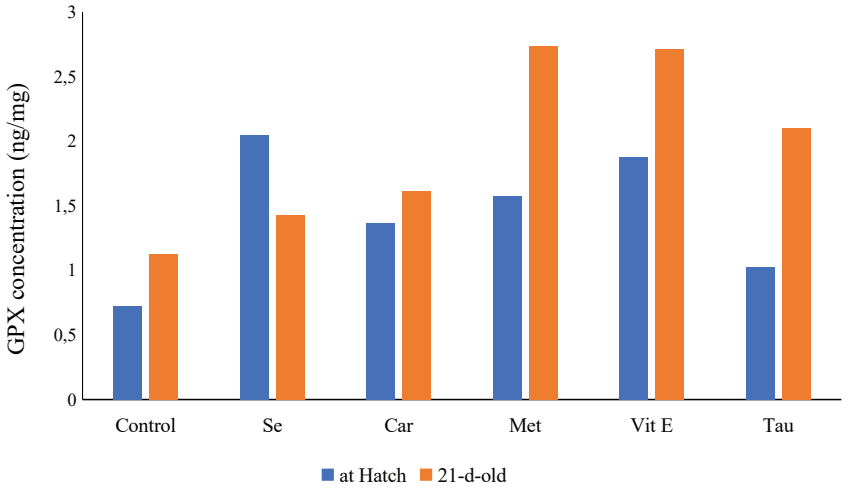

Figure 2. Comparison of GPX concentration in liver tissue of progeny between hatching and 21-d-old. *The differences between the means in the same group and at different ages are significant at the 0.05 level. **The differences between the means in the same group and at different ages are significant at the 0.01 level.

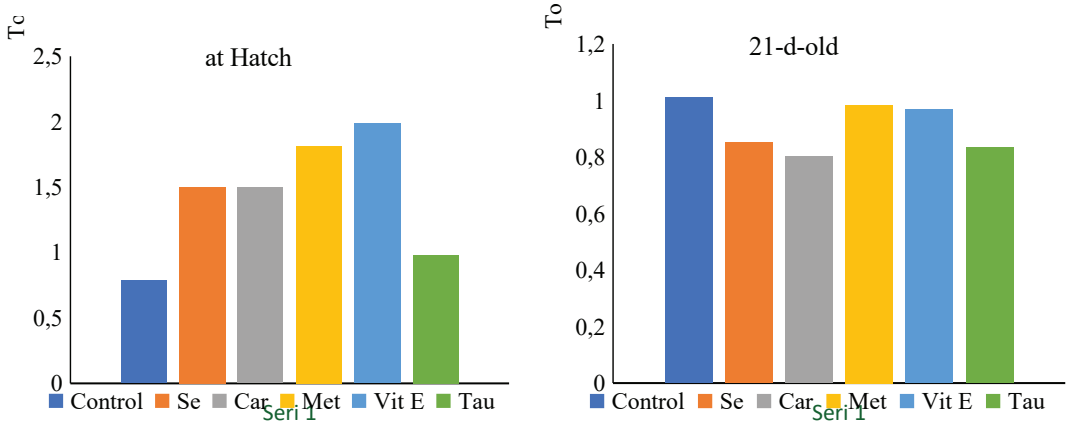

Figure 3. TOAC in liver tissue of progeny at hatching and 21-d-old. [abc]Different letters are significant($p<0.05$).

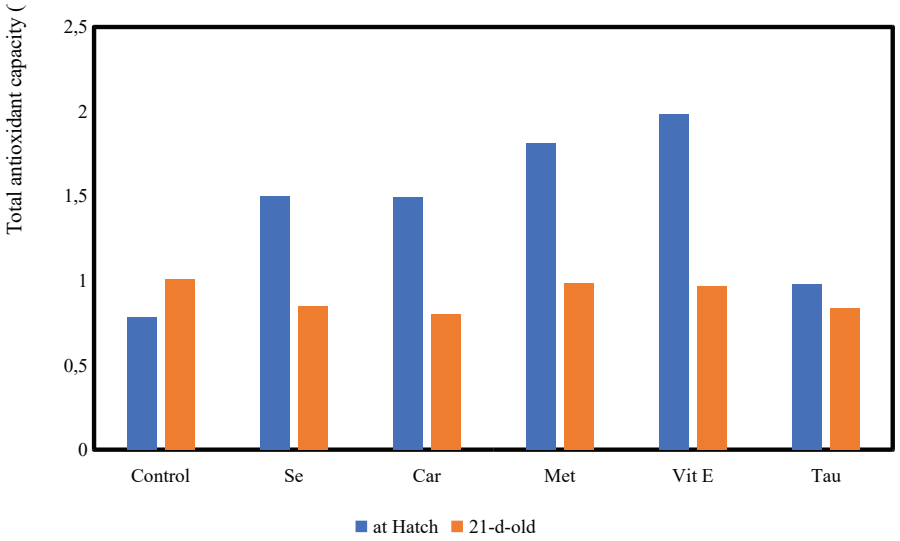

Figure 4. Comparison of TOAC in liver tissue of progeny between hatching and 21-d-old. *The differences between the means in the same group and at different ages are significant at the 0.05 level. **The differences between the means in the same group and at different ages are significant at the 0.01 level. ***The differences between the means in the same group and at different ages are significant at the 0.001 level.

Table 2. Effect of antioxidant supplementation on BW, FC, FCR, egg production, and egg weight of breeder quail (±SEM) (n=3)

	Week	Control	Se	Car	Met	Vit E	Tau	P-Value
BW (g)	Initial	226.9±1.60	227.3±2.48	231.0±1.05	225.6±2.33	234.8±5.32	227.6±4.79	0.344
	1st	230.9±2.37	231.5±2.38	233.4±3.49	231.1±0.36	234.5±2.11	230.7±4.31	**0.943**
	2nd	234.3±2.04	234.7±2.71	238.7±3.55	235.8±2.04	238.2±2.74	233.2±4.92	**0.785**
	3rd	243.4±2.54	237.7±2.76	243.6±3.91	240.2±0.60	242.1±2.75	236.1±6.75	**0.624**
	4th	237.5±2.43	239.9±2.66	242.3±4.12	240.1±0.48	239.3±6.00	238.2±6.02	**0.948**
FC (g)	1st	181.9±2.97	182.2±5.18	194.2±12.95	184.3±2.66	184.4±3.63	180.0±4.91	**0.622**
	2nd	188.8±4.56	184.4±4.67	196.9±5.80	191.2±1.13	196.9±2.63	183.6±5.70	**0.504**
	3rd	194.9±4.46	175.5±13.59	190.3±8.65	183.6±7.49	183.8±3.54	176.9±14.25	**0.412**
	4th	190.5±4.35	190.7±4.22	183.2±4.86	185.1±0.51	188.1±5.70	190.2±3.67	**0.880**
EP (%)	1st	85.1±1.56	86.8±1.85	85.0±3.92	81.9±4.47	81.5±2.93	83.9±4.36	**0.827**
	2nd	91.0±1.49	88.3±0.32	89.1±5.01	90.6±4.46	87.7±1.53	87.8±1.61	**0.863**
	3rd	88.6±2.03	76.9±12.88	88.2±2.68	86.0±5.71	85.9±2.51	81.0±12.36	**0.723**
	4th	85.2±2.25	90.2±2.35	88.6±4.16	92.9±3.50	89.7±2.43	90.5±1.65	**0.423**
EW (g)	1st	12.4±0.20	12.4±0.18	11.4±0.36	12.2±0.28	11.9±0.25	11.9±0.31	**0.139**
	2nd	11.9±0.32	12.6±0.43	11.9±0.38	12.2±0.22	12.8±0.32	12.0±0.31	**0.306**
	3rd	12.8±0.26	11.6±0.30	12.4±0.34	11.9±0.41	12.3±0.26	12.5±0.25	**0.102**
	4th	12.5±0.47	12.5±0.25	12.2±0.34	13.1±0.26	12.6±0.37	12.3±0.43	**0.641**
FCR	1st	2.5±0.06	2.4±0.07	2.9±0.34	2.7±0.11	2.4±0.11	2.6±0.12	**0.222**
	2nd	2.5±0.05	2.4±0.06	2.7±0.24	2.5±0.14	2.5±0.01	2.5±0.03	**0.557**
	3rd	2.5±0.06	2.9±0.34	2.5±0.19	2.6±0.07	2.5±0.08	2.6±0.22	**0.331**
	4th	2.6±0.05	2.4±0.03	2.4±0.17	2.2±0.08	2.4±0.07	2.4±0.03	**0.054**

Se: selenium group, Car: carnitine group, Met: methionine group, Vit E: vitamin E group, Tau: taurine group, BW: body weight, BWG: body weight gain, EP: egg production, EW: egg weight, FC: feed consumption, FCR: feed conversion ratio, and SEM: standard error mean.

Table 3. Effect of antioxidant supplementation on hatchability results

Groups	Control	Se	Car	Met	Vit E	Tau
Hatchability of total eggs (%)	85.5^{a}	85.5^{a}	86.0^{a}	82.5^{ab}	87.0^{a}	77.5^{b}
Fertility (%)	94.0^{ab}	94.5^{ab}	93.0^{ab}	94.0^{ab}	96.5^{a}	91.5^{b}
Hatchability of fertile eggs (%)	90.96^{ab}	90.48^{ab}	92.47^{a}	87.77^{ab}	90.16^{ab}	84.70^{b}
Early mortality (%)	2.13	2.65	2.15	2.66	4.15	4.37
Mid-term mortality (%)	2.13^{bc}	0.00^{a}	1.61^{abc}	1.06^{ab}	2.07^{bc}	4.37^{c}
Late-term mortality (%)	2.66	4.76	3.23	4.26	2.60	4.92
Under-shell mortality (%)	2.13^{ab}	2.12^{ab}	0.54^{a}	4.26^{b}	1.04^{a}	1.64^{ab}

^{abc}There is statistical significance between different letters on the same row($p<0.05$). Se: selenium group, Car: carnitine group, Met: methionine group, Vit E: vitamin E group, and Tau: taurine group.

Table 4. Effect of maternal antioxidant supplementation on BW, BWG, FC, and FCR of progeny (±SEM) (n=4)

	Day	Control	Se	Car	Met	Vit E	Tau	P-Value
BW (g)	1	7.7±0.08	7.6±0.09	7.5±0.12	7.8±0.08	8.0±0.16	8.1±0.30	**0.073**
	7	25.8±0.73	25.5±1.11	24.6±1.29	25.1±1.53	24.2±1.14	23.5±2.29	**0.879**
	14	63.0±0.71	63.0±0.53	63.0±1.93	64.2±2.49	63.0±0.71	60.7±3.42	**0.889**
	21	107.3±3.93	109.1±4.67	105.0±2.21	106.0±3.52	103.3±0.54	104.2±3.51	**0.841**
	28	152.9±2.62	145.2±0.54	147.7±4.47	150.5±1.71	148.0±3.51	144.7±3.44	**0.407**
BWG (g)	1-7	18.1±0.70	17.9±1.13	17.1±1.18	17.2±1.50	16.3±1.16	15.4±2.57	**0.792**
	8-14	37.2±0.24	37.6±1.09	38.4±1.18	39.1±1.02	38.8±1.25	37.2±1.50	**0.743**
	15-21	44.3±3.22	46.1±4.56	42.0±0.93	41.8±1.91	40.3±0.58	43.5±0.51	**0.632**
	22-28	45.6±2.00	36.1±4.56	42.8±2.39	44.6±2.21	44.7±3.62	40.5±0.15	**0.231**
FC (g)	1-7	23.3±0.95	25.0±1.38	27.2±1.17	23.9±2.91	23.1±0.74	24.3±1.86	**0.542**
	8-14	69.1±3.83	68.2±3.37	70.6±4.84	68.4±1.69	62.6±5.69	72.1±2.79	**0.639**
	15-21	96.5±5.47	94.7±3.29	92.1±1.77	94.3±2.51	90.6±0.60	96.6±5.74	**0.832**
	22-28	119.0±5.47	116.9±2.98	103.0±11.81	100.9±2.00	107.8±3.83	113.5±5.73	**0.378**
FCR	1-7	1.3±0.02	1.4±0.08	1.6±0.13	1.4±0.08	1.4±0.07	1.4±0.03	**0.145**
	8-14	1.9±0.09	1.8±0.11	1.8±0.12	1.8±0.02	1.6±0.13	2.0±0.11	**0.333**
	15-21	2.2±0.12	2.1±0.20	2.2±0.03	2.3±0.06	2.3±0.02	2.2±0.12	**0.934**
	22-28	2.6±0.13	2.9±0.12	2.4±0.30	2.3±0.09	2.4±0.13	2.8±0.14	**0.142**

Se: selenium group, Car: carnitine group, Met: methionine group, Vit E: vitamin E group, Tau: taurine group, BW: body weight, BWG: body weight gain, FC: feed consumption, FCR: feed conversion ratio, and SEM: standard error mean.

RESULTS

The performance results for breeder quail are given in Table 2. The difference between the control, carnitine, selenium, methionine, vitamin E, and taurine breeder groups was statistically insignificant in terms of body weight ($p>0.05$). Also, feed consumption, egg production, egg weight, and feed conversion ratio were insignificant among all groups ($p>0.05$).

The results of incubation are given in Table 3. The lowest hatchability of total eggs, fertility, and hatchability of fertile eggs was determined in the Tau group ($p<0.05$). The differences between the experimental groups regarding early and late-term embryo mortality were insignificant ($p>0.05$). Mid-term embryo mortality was highest in the Tau group ($p<0.05$). In addition, mid-term embryo mortality did not occur in the Se group. While under-shell mortality was highest in the methionine group at 4.26%, the lowest was in the Car group at 0.54% ($p<0.05$).

During the chick growing period, the weekly averages for BW, BWG, FC, and FCR are given in Table 4. The effect of antioxidant supplementation in breeder feeds on BW, BWG, FC, and FCR of chicks was insignificant ($p>0.05$).

GPX enzyme concentration in the liver tissues of progeny was highest in the Se, Vit E, and Met groups with values of 2.044, 1.875, and 1.575 ng/mg, respectively (Figure 1), while it was lowest in the control group at 0.726 ng/mg ($p<0.05$). The differences between the Car, Tau, and control groups were insignificant ($p>0.05$), but the differences between the control and the other experimental groups were significant ($p<0.05$) in the liver tissues of 1-d-old progeny.

The methionine group had the highest concentration of GPX in liver tissues of 21-d-old progeny (2.739 ng/mg) (Figure 1), whereas the control group had the lowest value (1.127 ng/mg) ($p<0.05$).The differences between the control, Se, and Car groups were not statistically significant ($p>0.05$); however, there were significant differences between the control group and the Met, Vit E, and Tau groups ($p<0.05$).

The GPX concentrations in control, Met, Vit E, and Tau groups were significantly higher in 21-d-old progeny compared to 1-d-old progeny (Figure 2) ($p<0.05$). However, the effect of age on GPX concentration was insignificant in the Se and Car groups.

TOAC in the liver tissues of 1-d-old chicks was highest in the Vit E group at 1.987 U/mg (Figure 3), while the lowest value was 0.786 U/mg in the control group ($p<0.05$). The difference between the control and Tau groups was insignificant ($p>0.05$); however, the differences between the control and the other

groups were significant ($p<0.05$).

There was no significant difference among treatments concerning TOAC in the liver tissues of 21-d-old progeny (Figure 3) ($p>0.05$).

TOAC in the liver of 1-d-old chicks was higher in all groups, except control and Tau groups, when compared to 21-d-old chicks (Figure 4) ($p<0.05$).

An increase in the expression level of a gene indicates that the gene is upregulated, while a decrease in the expression level indicates that it is downregulated. The expression levels of the GPX1 gene in all experimental groups were lower than the control group (Figure 5). While the group with the lowest expression level was the Tau group, this group was followed by the Met, Vit E, Car, and Se groups, respectively. GPX1 gene was downregulated by 0.9 in Se, 0.65 in Car, 0.5 in Met, 0.55 in Vit E, and 0.45 in Tau group compared to the control group for 1-d-old progeny.

GPX1 gene was downregulated by 0.95 in Se and 0.75 in Met groups compared to the control group for 21-d-old progeny. GPX1 gene was upregulated by 1.48 in Car, 1.10 in Vit E, and 1.75 in Tau groups compared to the control group for 21-d-old progeny (Figure 6).

The expression level of the GPX1 gene in the liver tissue of all 1-d-old progeny groups was higher than in the liver tissue of 21-d-old quail chick (Figure 7).

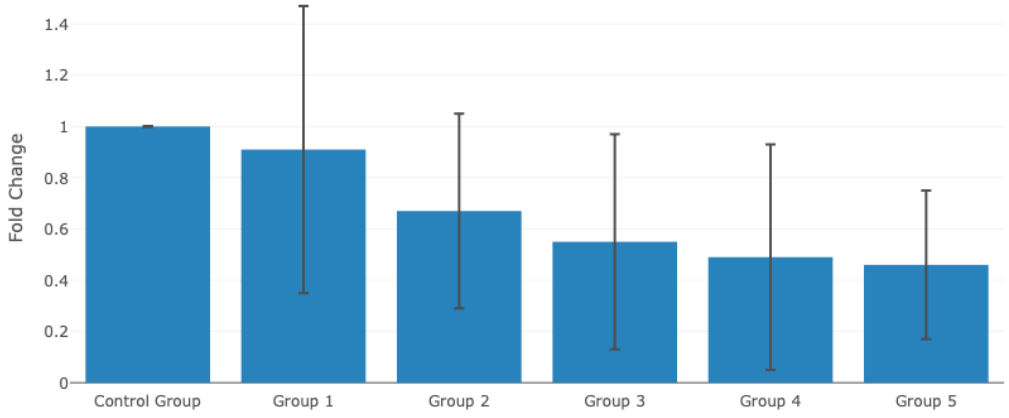

Figure 5. Expression levels of GPX1 gene in liver tissue of1-d-old chicks. Group 1: Se group, Group 2: Car group, Group 3: Vit E group, Group 4: Met group, and Group 5: Tau group.

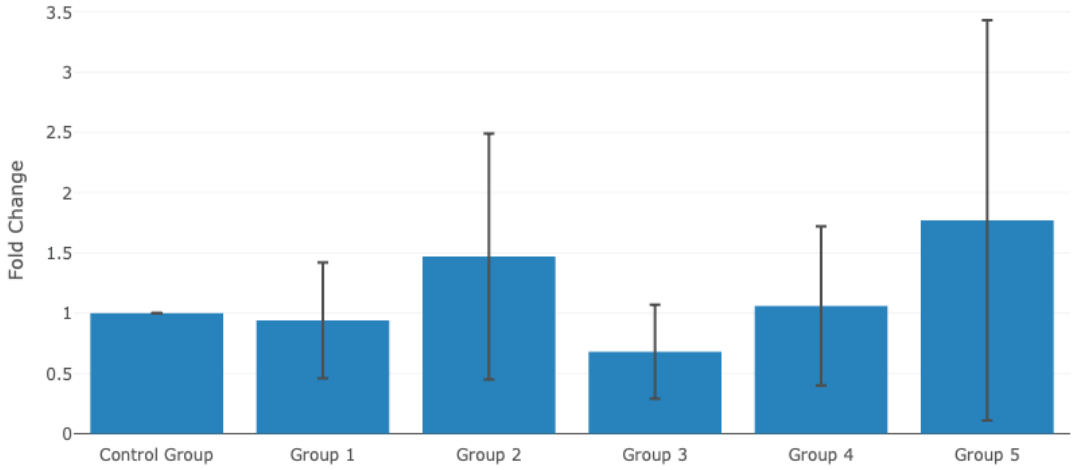

Figure 6. Expression levels of GPX1 gene in liver tissue of 21-d-old progeny. Group 1: Se group, Group 2: Car group, Group 3: Met group, Group 4: Vit E group, and Group 5: Tau group.

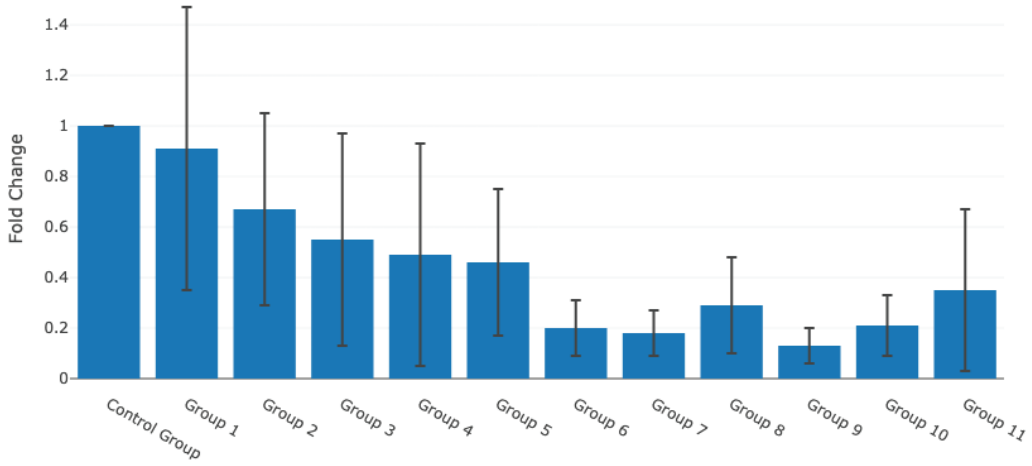

Figure 7. Comparison of expression levels of GPX1 gene in liver tissue of progeny between hatching and 21-d-old. Control Group: Control group at hatching, Group 1: Se group at hatching, Group 2: Car group at hatching, Group 3: Vit E group at hatching, Group 4: Met group at hatching, Group 5: Tau group at hatching, Group 6: Control group at 21-d-old, Group 7: Se group at 21-d-old, Group 8: Car group at 21-d-old, Group 9: Met group at 21-d-old, Group 10: Vit E group at 21-d-old, and Group 11: Tau group at 21-d-old.

DISCUSSION

The diet of the breeder quails used in the study was enhanced in terms of vitamin E, methionine, carnitine, taurine, and selenium contents, respectively. Adding these antioxidants to the breeder quail rations did not affect body weight, feed consumption, egg production, egg weight, and feed conversion ratio.

There were no differences between the control and experimental groups regarding fertility and the hatchability of fertile eggs. Augmenting Se in the diet reduced mid-term mortality while adding carnitine reduced under-shell mortality. The embryo, which has low oxygen consumption in the early period of incubation, increases oxygen consumption in the middle period of incubation with the development of lung respiration and is exposed to damage from ROS, such as hydrogen peroxide superoxide radicals, and hydroxyl radicals (Fu et al., 1999; Li et al., 2020). GPX is involved in removing lipid hydroperoxides and hydrogen peroxide formed during metabolism and dismutation of superoxide radicals and needs Se as a cofactor (Surai, 2000). The energy requirement of the embryo developing in the egg increases daily depending on body weight and reaches a peak level in the last stages of incubation. The lack of energy during this period can cause enteric developmental disorders (Ferket, 2006) and even death. During the last week of incubation, beta-oxidation of fatty acids constitutes the primary energy source for the embryo (Uni et al., 2012). Carnitine acts as an emergency carrier for beta-oxidation of long-chain fatty acids (Agren et al., 2014) and thanks to its antioxidant feature, it both supports energy metabolism and protects the organism from damage caused by ROS.

Supplementation of Se, carnitine, methionine, vitamin E, and taurine in breeder quail feed did not affect BW, BWG, FC, and FCR of progeny because the basal feeds used in the experiment contained the nutrients needed by the animals.

In this study, the GPX enzyme concentrations at hatching were ranked control≤taurine≤carnitine≤methionine≤vitamin E≤selenium group, while at 21-d-old the ranking was: control≤selenium≤carnitine≤taurine<vitamin E≤methionine group. This study revealed that supplementing different antioxidants into quail breeder diets significantly affected glutathione peroxidase enzyme activity, especially supplementation with methionine and vitamin E compared to chicks fed supplementation with Se and control feed at the 3rd week of age. Supplementation with methionine (Kalvandi et al., 2019), Se (Karadas et al., 2004; Nassef et al., 2020), and vitamin E (Sarıca et al., 2019; Shah et al., 2016) in Japanese quail feed increased the concentration of GPX in blood or liver tissue. Karadas et al. (2004) reported that the Se level in the liver tissue of chicks was 885.4 ng/g at hatching, 326.3 ng/g at 7-d-old, and 149 ng/g at 14-d-old in a study in which 0.5 ppm organic Sel-Plex was added to quail breeder feeds. The addition of Se to breeder feeds significantly increased the liver Se concentration for 14 days after hatching and affected the anti-

oxidant system (Surai, 2006). The results of this study are in agreement with the above studies.

Antioxidants added to breeder quail feeds increased TOAC in the liver of quail chicks at hatching, but the same effect was not seen on the 21st day. Therefore, it is understood that the effect of antioxidant addition to breeder feeds on TOAC in quail chick liver tissue is valid for a limited time. Hatching is regarded as an environmental stress or for the chicks, and at this time, the natural antioxidant concentrations in tissues reach their maximum level (Surai, 2007). In addition, high temperature, humidity, and unsaturated fats in tissues increase the risk of lipid peroxidation during this period. High TOAC in tissues at hatching plays a vital role in protecting chicks against lipid peroxidation.

All antioxidants downregulated GPX1 gene expression in liver tissues of 1-d-old quail chicks. Se and methionine treatments downregulated GPX1 gene expression, while carnitine, vitamin E, and taurine treatments upregulated GPX1 gene expression in the liver tissue of 21-day-old quail chicks. In addition, the expression level of the GPX1 gene in the liver tissue in all experimental groups was higher at 1-day-old than at 21-d-old. Liu et al. (2012) reported that the supplementation of Se to feed significantly increased the expression level of the GPX1 gene in liver and kidney tissues, in their study where they fed pigs with selenium-deficient feed and added 0.3 mg/kg or 3 mg/kg selenium-enriched yeast to this feed in other groups. Adding 3 mg/kg selenium yeast to the diet decreased the expression level of the GPX1 gene insignificantly compared to 0.3 mg/kg selenium yeast supplementation in these tissues. Since the GPX1 gene is in the selenium-dependent gene group, GPX1 gene expression level remained low in animals fed a selenium-deficient diet. Wilaison and Mori (2009) reported that adding different doses of selenium (0.25, 0.5, and 1 ppm sodium selenite) to the basal feed of Japanese quail (containing 0.24 ppm Se) increased GPX1 enzyme expression levels compared to the control group in the liver tissues of 1-d-old chicks. Since the selenium source used in this study and the selenium doses added to the feeds differed from the current study, GPX1 expression results in the liver tissues of day-old chicks were not similar. In a study of broilers by Del Vesco et al. (2015), heat stress and high methionine content in feeds increased glutathione peroxidase 7 (GPX7) gene expression in breast tissue. In addition, when the methionine level in the feed is insufficient or high, the gene expression levels decrease compared to sufficient methionine level. This result showed that animals exposed to high-temperature stress try to prevent the increase in ROS production by increasing the expression level of the GPX7 gene, which is part of the glutathione-dependent antioxidant system (Del Vesco et al., 2015).

Glutamate, glycine, and cysteine are used to synthesize glutathione. Methionine, the precursor of homocysteine, may be used to create cysteine (Shoveller et al., 2005). Therefore, if dietary methionine is sufficient, the remaining methionine plays a role in cysteine synthesis via transsulfuration. The impact of taurine and heat stress on gene expression levels in Japanese quails was examined by Orhan et al. (2020). Taurine did not affect gene expression levels without heat stress but increased gene expression levels under heat stress. The increase in mRNA levels of antioxidant enzyme genes is an indicator of the activation of the antioxidant system (Wang et al., 2019; Zheng et al., 2016). Yarru et al. (2009) reported that the addition of turmeric (*Curcuma longa*) powder (TMP) to feed containing aflatoxin B1 increased the expression level of the GPX gene in the liver tissues of the broiler. Thus, adding TMP to animal feed improved antioxidant protection and converted the highly reactive hydrogen peroxide (H2O2) to water (H2O). The addition of 12% glycerin to broiler feeds increased the expression level of the GPX gene in broiler liver tissue 4 times compared to the livers of those receiving feed without glycerin (Araújo et al., 2018). Adding 12% glycerinto feed reduced the oxidative stress associated with peroxide activity during oxygen metabolism in the cells of this organ. Stress caused changes in the gene expression level, so there was a relationship between supplementing antioxidants in the diet and stress on gene expression. In our study, TOAC and GPX enzyme concentrations in the liver tissue of 1-d-old chicks increased with the addition of antioxidants to the feed, and a stronger antioxidant defense mechanism might have developed. Since the organism was not exposed to any stress and the antioxidant defense capacity was sufficient, the GPX1 gene expression was not affected in the liver. However, the expression of the GPX1 gene was upregulated in the carnitine and taurine groups compared to the control group at 21-d-old, and thus, the birds in these groups had better antioxidant defense mechanisms.

In conclusion, the addition of antioxidants to breeder feed positively affected the hatchability of total eggs, fertility, embryonic mortality, and GPX enzyme

concentration and TOAC in liver tissues of progeny. In addition, no adverse effects were observed on BW, BWG, FC, and FCR of progeny. The GPX1 gene expression in the liver tissues of 1-day-old quail chicks was downregulated by all antioxidants. In the liver tissue of 21-day-old quail chicks, treatments with taurine, carnitine, and vitamin E upregulated GPX1 gene expression, while treatments with selenium and methionine downregulated it.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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LIST OF ABBREVIATIONS

BW: body weight, BWG: body weight gain, C: control, Car: carnitine, CAT: catalase, cm: centimeter, FC: feed consumption, FCR: feed conversion ratio, g: gram, GPX: glutathione peroxidase, GPX1: glutathione peroxidase 1, GPX7: glutathione peroxidase 7, GSH: glutathione, ID: iodothyronine deiodinase, kg: kilogram, LPO: lipid peroxidation, MDA: malondialdehyde, Met: Methionine, mg: milligram, NF-B: nuclear factor-B, ng: nanogram, Nrf2: nuclear factor erythroid 2-related factor 2, ppm: parts per million, ROS: reactive oxygen species, Se: Selenium, SeMet: selenomethionine, SOD: superoxide dismutase, TAOC: total antioxidant capacity,Tau: Taurine, TMP: turmeric powder, TrxR: thioredoxin reductase,U: Unit, Vit E: vitamin E, °C: degree centigrade, and %: percent.

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